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Genetic Insight: PON1-C(L55)/T Polymorphism Elevates Cardiovascular Risk in Women with PCOS

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KEYWORDS
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ABSTRACT

Paraoxonase 1 (PON1) is a high antioxidant density (HDL) related enzyme that plays an important function in preventing oxidation of low antioxidant density (LDL) and HDL. PON1 activity have recently been investigated in order to determine its link to cardiovascular disease, diabetes, cancer, infertility, and other conditions. The PON1 gene's variation may influence metabolism, resulting in an imbalance of pro-oxidants and antioxidants. This enzyme's critical involvement may put polycystic ovarian syndrome (PCOS) women at risk for cardiovascular disease. This study looked at samples from 56 healthy women and 53 women with PCOS. The PON1 L55 C/T frequencies were determined using PCR and RFLP experiments. To see if there was a link between the polymorphism and cardiovascular risk, researchers looked at the enzyme activity and polymorphism. Women with the L55 TT genotype (as opposed to L55 C alleles) had lower paraoxonase activity in PCOS patients and controls. The C/T polymorphism in PON1 L55 has been linked to PCOS in women. As a result, it could be a key driver of cardiovascular risk in women with PCOS.

INTRODUCTION

Cardiovascular disease is the most common disease which may lead to death in both men and women (1). Some common signs that are risk factors are elevated blood pressure, obesity, smoking, diabetes mellitus etc. The primary cause of death in women is heart disease but it is often underestimated that they are protected against cardiovascular disease (1,2). There may be many reasons for women getting CVD but one of the main reasons is due to metabolic reactions associated with polycystic ovarian syndrome (PCOS). Diabetes and Obesity are some common symptoms for CVD and PCOS (3-6). There is much evidence stating that women with PCOS are observed with coronary artery disease and myocardial infarction (7-9). PCOS is a condition which shows symptoms like infertility, irregular menstrual cycle, oxidative stress in premenopausal women. Sudden increase in oxidative stress leads to CVD in women with PCOS (10). Many experiments and evidence from studies shows that paraoxonase-1, calcium dependent esterase, is bound to HDL-C. It is also recognized as an antioxidant enzyme as it hydrolyses lipid peroxides in oxidized lipoprotein (11,12). The PON1 gene was identified as a cluster of enzymes having organophosphates as substrates. It stopped the harmful substances from causing cellular damage, which revealed the genetic polymorphisms (13,14). PON1 gene's variability affects its expression and activity, which in turn affects the susceptibility of women to PCOS (15). While screening the PON1 gene, the functional single nucleotide polymorphisms (SNPs) are analysed. It shows that C(L55)T is located in the promoter and coding sequence of the paraoxonase gene (16,17). This study aims to understand enzyme activity and prove the association of L55C//T and CVD risk.

Subjects:

Samples of 108 individuals were collected, 53 cases with PCOS recruited from the unit of gynaecology and obstetrics at Sri Ramachandra Medical and Research institute, Porur, Chennai. To conform to the PCOS, the ultrasonographic examination was done (Fig.1(b)). PCOS causes insufficient flow, infertility, obesity and dysfunctional bleeding. Controls were done for 56 cases with normal ovaries and regularly menstruating women with the same age (Fig.1(a)). The study follows all ethical guidelines and all written consent was obtained.

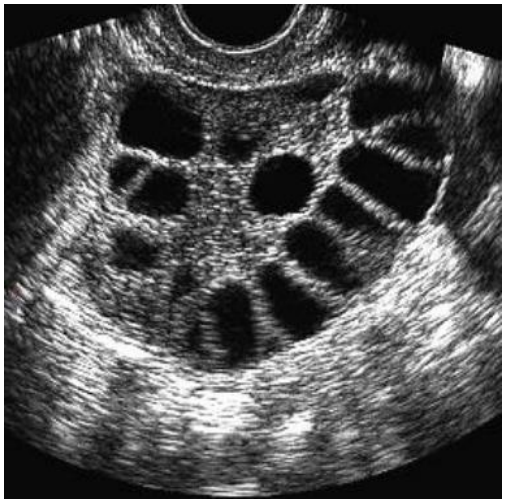

Fig. 1(a): The ultrasonography examination of Normal Ovary

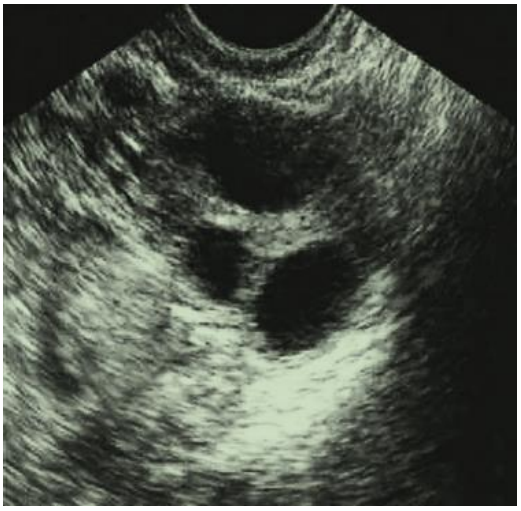

Fig. 1(b): The ultrasonography examination of PCOS Ovary

2. **MATERIALS AND METHODS:**
For the test and control subjects, 5 ml of blood samples were collected and stored at -20 ˚C for further analysis. Fors biochemical studies the serum was separated. The DNA was isolated from the blood for genetic study. From reputed vendors like SRL, star labs, tarsons, the plastics, glass wares were purchased and the chemicals used were analytically graded.

2.1 Determination of lipid profile
To determine the lipid profile, the collected blood samples were used. By performing Parekh and Jung method (1970) (18) cholesterol was estimated. By performing the method of Rice (1973) (19) triglycerides were estimated. By precipitation of apolipoprotein with heparin manganese chloride reagent by Eisenberg method (1984) (20) HDL- cholesterol was determined by Wilson and Spiger method (1973) (21), LDL- cholesterol was determined.

2.2 Estimation of Paraoxonase
To determine the activity of paraoxonase, spectrophotometric assay was done with paraoxon (O, O-Diethyl-O-P-nitrophenyl-phosphate). Based on the test conditions, one unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute.

2.3 Genotypic analysis
For genomic analysis, to extract the genomic DNA, modified Miller's protocol was used. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was done for C(L55)/T genomic analysis. To analyse the genotype of C(L55)/T, primers were designed (Table.1).

In PCR following steps were performed, initial denaturation at 94℃ for 5 minutes followed by 30 cycles of denaturation at 94℃ for 1 minute, annealing at 51.1℃ for 30 seconds, followed by extension at 72℃ for 1 minute then after 30 cycles the final extension at 72℃ for 6-8 minutes.

With the specific restriction enzyme BsrBI , the PCR amplicons were incubated at 37℃ for 2 hours. By gel electrophoresis, the digested PCR Amplicons were transferred and allowed for data analysis.

No.	Primer Name	Primer Sequence	Primer Length	GC%	Tm	Product Size
	PC1	5' CCT GCA ATA ATA TGA AAC AAC CTG 3'	24 mer	37.5%	57.6˚C	172 bp
2.	PC2	5' TGA AAG ACT TAA ACT GCC AGT C 3'	22 mer	40.9%	56.5˚C	172 bp

Table 1: Details of Primers designed

3. **RESULTS**:
The results show that the participants have a different lipid profile and are dyslipidemic. The current investigation discovered a considerable increase in triglycerides. (Table.2). It was also discovered that HDL levels have decreased little, however LDL levels have increased significantly. The build-up of triglycerides could be due to increased lipogenesis, slower clearance, or reduced fatty acid oxidation. Excessive metabolites such as diacylglycerols, fatty acids, and ceramides can result from an excess of triglycerides. As a result, it contributes to the risk of Cardiovascular disease CVD by causing fat accumulation in non-adipose tissues. The current study discovered a decrease in the amount of the paraoxonase enzyme, which could have a role in the development of AHD in PCOS patients.

The genotype and allele frequencies between PCOS individuals and the control group were examined for L55M polymorphism (Fig. 2). Figures 3 and 4 shows RFLP reaction for the L55M polymorphism research and its restriction digestion pattern using PC3 and PC4. The Hardy Weinberg Equilibrium was established, and the charts showed the genotypic and allelic frequency distribution. The M allele's Minor Allele Factor (MAF), which rises from 17.9% to 27.4% from control to PCOS participants (Tables 3.1 and 3.2), shows a weak connection with PCOS subjects (Fig. 5(a)). Fig. 5(b) displays graphically the allele frequency distribution between PCOS and control. To accurately determine the relationship between the L55M polymorphism (rs854560) in the PON1 gene and polycystic ovarian syndrome, larger-scale research is needed. According to (Mohammed et al., 2009) study (22), PON1 -108 C/T and L55M polymorphisms may be associated with PCOS

in Egyptian women and may have an impact on the insulin resistance index and serum paraoxonase levels.

S. No	Parameters	Control	Test	F VALUE	P VALUE
1.	Cholesterol	174.8022 ± 10.8701	212.4630 ± 18.8243	13.248	0.0000**
2.	Triglycerides	108.3022 ± 22.3991	177.1304 ± 16.8900	4.723	0.032*
3.	HDL	57.4543 ± 5.0144	38.5637 ± 7.02422	2.604	0.110
4.	LDL	106.7261 ± 9.8432	131.8696 ± 22.3991	3.655	0.059

Table 2: Dyslipidaemia status of subjects

Genotypes	Control (%)	Case (%)	P value*
Homozygote reference (LL)	67.9%	47.2%	0.0324
Heterozygote (LM)	28.6%	50.9%	0.6899
Homozygote variant (MM)	3.6%	1.9%	0.7004
MAF*	**17.9%**	**27.4%**	**0.1292**

MAF – Minor Allele Frequency

Table 3.1: Percent and gene frequencies of L55M Polymorphism of PON1 gene

Case vs. Control	ODDs Ratio	P value**	95 % CI	
LM vs. LL	2.36	0.04640	1.086	5.148
MM + LM vs. LL	2.36	0.04640	1.086	5.148
M vs. L	1.29	0.08825	0.665	2.507
		**Unadjusted value		

Table 3.2: Odd ratio of L55M Polymorphism of PON1 gene

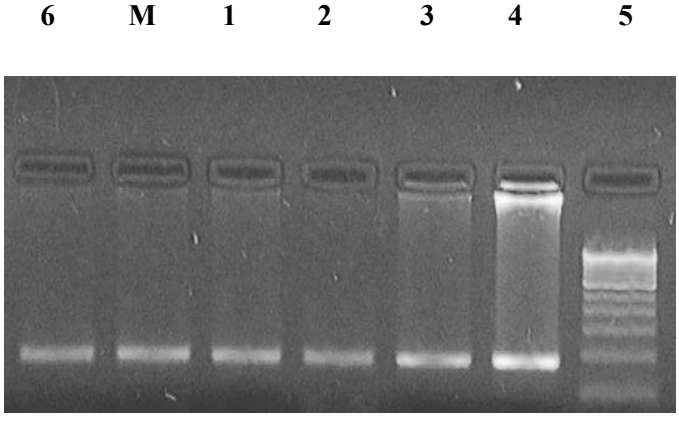

Fig. 2. PCR Reaction for L55M Polymorphism

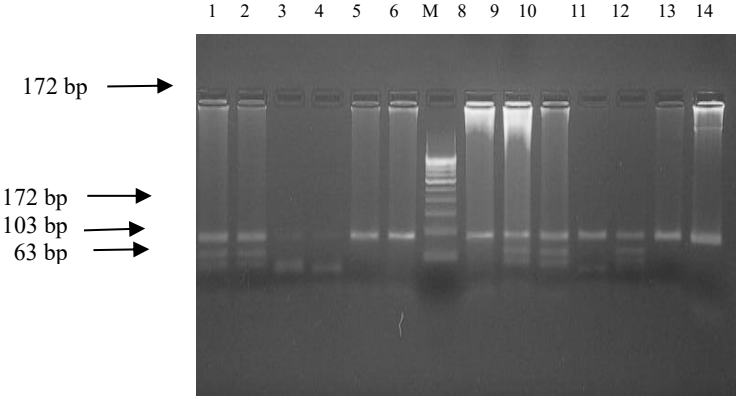

Fig. 3. RFLP Reaction for L55M Polymorphism

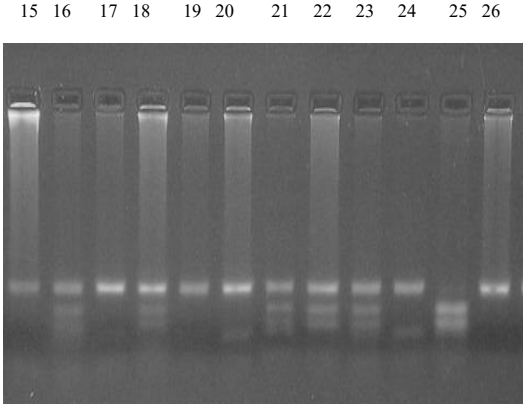

Fig. 4. Restriction Digestion Pattern of L55M Polymorphism

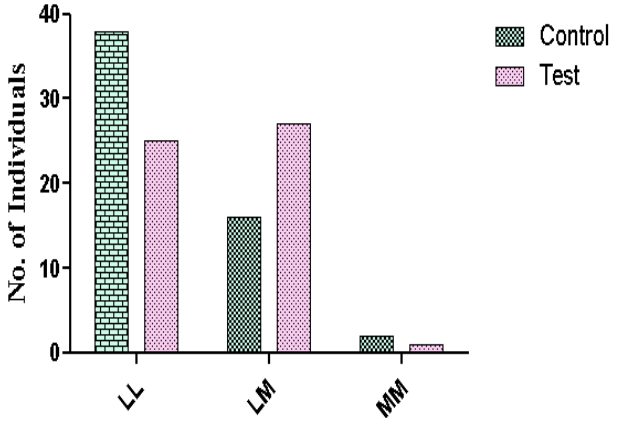

Fig. 5(a). Genotype frequency of L55M Polymorphism

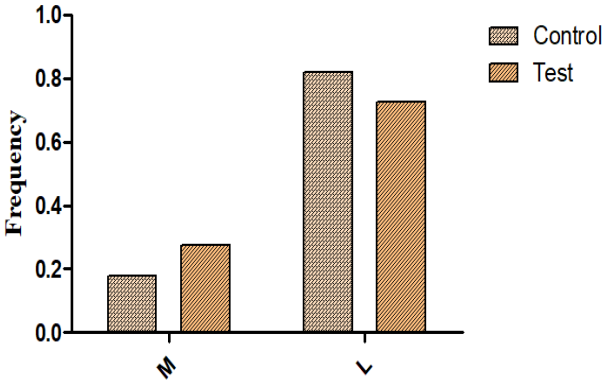

Fig. 5(b). Allele frequency of L55M Polymorphism

DISCUSSION

Oxidative stress is associated with a number of illnesses, including aging and aging-related chronic diseases such diabetes mellitus, atherosclerosis, and ischemia reperfusion injury. According to a recent study, increased oxidative stress and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in women with PCOS, in addition to known risk factors such insulin resistance, hypertension, central obesity, and dyslipidemia (23,24). These patients must maintain a healthy diet and antioxidant supplements like vitamin C and vitamin E in order to develop an adequate antioxidant defence system and reduce the harmful effects of excessive oxidative stress. Oxidative stress complicates the genesis of insulin resistance and cardiovascular disease (10,25). In addition to interfering with insulin function, oxidative stress results in chronic inflammation, which aggravates atherosclerosis, heart disease, and type 2 diabetes. Furthermore, the pathophysiology of PCOS is mostly driven by chronic inflammation. Women with PCOS have decreased serum PON1 activity and are more likely to develop atherosclerosis (26,27). In our study, the PCOS group had lower blood levels of PON1 activity than the other groups. Androgens and proinflammatory mediators reduce liver PON1 expression, which is influenced by genetic and environmental factors. The serum paraoxonase gene, PON1, is primarily expressed in the liver. In addition, some studies have found that PON1, dyslipidaemia, and insulin resistance increase the risk of cardiovascular disease in PCOS affected women (28). Therefore, using medications that alter these characteristics can aid PCOS patients in avoiding long-term health effects.

CONCLUSION

This study analysed the polymorphism of the gene and the enzyme activity of Paraoxonase 1 (PON1). The Paraoxonase 1 (PON1) enzyme's notable distinction may lead to PCOS women developing CV disease risk factors. Consequently, there is a connection between enzyme activity, the PON1 L55M polymorphism, and risk elements associated with the aberrant profile in PCOS patients. As a result, these patients may be at risk for CV illnesses.

COMPETING INTERESTS:

No conflicts of interests

DATA AVAILABILITY STATEMENT:

The sequence is not reported in any of the previous studies. So, the data availability statement is not applicable.

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